SURFACE MODIFICATION FOR BIOCOMPATIBILITY

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TABLE OF CONTENTS

	Page
FIGURE AND TABLE CAPTIONS	ii
PROJECT SUMMARY	1
QUARTER OBJECTIVES	2
BACKGROUND	3
RESULTS	4
NEXT QUARTER OBJECTIVES	6
REFERENCES	6

FIGURE CAPTIONS

Figure 1: Phase images of cortical neurons derived from mature adult rat cultured under serum-free conditions with addition of 5 ng/ml bFGF at 1 and 4 days *in vitro*.

Figure 2: Bar graphs diagramming the effects of b-FGF on survival of astrocyte cultures *in vitro* at 1, 4 and 7 days. The addition of b-FGF lends a "protective" effect, enhancing attachment and survival on traditionally "poor" surfaces (13F, OTS, TP) with little change to adhesion and survival on characteristically "good" surfaces (PEDA, DETA, PL).

Figure 3: Phase (1, 3, 5) and fluorescence (2, 4, 6) microscopy showing microglial cultures and differential adhesion according to substrate. Both ameboid and activated stages of microglia are present on control poly-D-lysine cultures (1, 2, 3, 4), with diminished attachment on DETA substrates (5, 6).

Figure 4: XPS survey Spectra. APTS monolayer (bottom). APTS monolayer derivatized with a covalent crosslinker, glutaraldehyde (middle). APTS monolayer with covalent crosslinker glutaraldehyde attached to apo-transferrin (top). The X-axis shows the binding energy of electrons in the film and is directly related to different elements. The Y-axis shows the relative XPS intensity. Note that the size of the C1s and N1s signal as compared to the size of the Si2p signal and is indicative of the amount of bound material after reaction and washing to remove loosly bound materials.

PROJECT SUMMARY

The aim of this work is to create surfaces on implantable silicon microstructures for the purpose of controlling the interaction of neurons, glia, and related cells and their protein products with the microstructure. A major effort in previous work was devoted to the examination of culture conditions for early embryonic cultures with an aim to promote longevity in culture. We have established a serum-free culture system that approximates the composition of cerebral spinal fluid (CSF) for culturing the embryonic cells and have now extended this work to adult cultures as well. For embryonic cells, the E16 cortical cultures survive to 21 days and the E19 cells survive to 19 days.

In this quarter we concentrated on repeating experiments with E14, astrocyte and microglial cultures for publication. We continue to examine the impact of substrate on neuronal subpopulations and neurotransmitter production (E14). Most importantly to the neural prothesis program we now have a reproducable *in vitro* model for cortical neurons from E14 to adult cells as well as the other cell types contained in the CNS such as astrocytes and microglia. This now allows us to test the effect of surfaces, factors, and insult in a reproducible defined system before *in vivo* experiments. We will concentrate on the adult culture experiments in the next quarter.

We have also determined these cells are responsive to various artificial monolayer surfaces and have proven strategies for microelectrode modification protocols. The results are being prepared for publication. Now we will extend the surface modifications to include biological macromolecules.

OBJECTIVES

Overall project objectives:

a) Selecting candidate surfaces that are likely to enhance the microscopic mechanical stabilization of a microstructure implanted within the central nervous system;

b) Selecting candidate organic surfaces that are likely to enhance the close approximation of neurons or neuronal processes to specific regions of implanted silicon microstructures;

c) Developing or adapting available methods to bond the selected organic molecules to a silicon dioxide surface like the surface of a micromachined electrode (Tanghe and Wise, 1992) and to chemically characterize these surfaces before and after protein adsorption.

   1. The attachment method shall be stable in saline at 37°C for at least 3 months;
   2. To use silane coupling as the method of attachment;
   3. To use the silanes to control the spatial extent (i.e., the pattern) of the deposited surface.

d) Developing a cell culture or other suitable model of mammalian cortex and investigate the growth and adhesion of neurons, glia, micro-glia, and other cells present in the nervous system on substrates coated with the selected surfaces;

e) Cooperating with other investigators in the Neural Prosthesis Program by coating microelectrodes (estimated 50 over the contract period) with the most promising materials for *in vivo* evaluation as directed by the NINDS Project Officer.

QUARTER OBJECTIVES

- Continue work to establish cortical cell culture conditions for optimal 4 week survival
- Begin screening biologically modified SAM surfaces for cortical cell survival
- Continue screening E14 response to artificial surfaces
- Continue screening PN10 response to artificial surfaces
- Continue surface analysis of surfaces both before and after culture
- Continue surface stability experiments in saline on SiOX/glass
- Continue screening surfaces for microglia response
- Send samples to Huntington for *in vivo* experiments as well as explore other possibilities for collaboration in this area

BACKGROUND

Biomaterials that penetrate into the central nervous system as the microscopic electrode shafts of neural prostheses interact with neural and other tissues on a cellular and molecular level. In order to achieve tight coupling between these implanted microelectrodes and the target neural substrate, this interaction must be understood and controlled. Controlling the interaction requires an understanding of how cells, including neurons and glia, and extracellular proteins respond to the surface chemistry and any leachable substances of implanted biomaterials. This contract supported research will study these interactions with a long-term goal of rationally designing microelectrode surfaces to promote specific tissue interactions.

Presently, available clinical neural prosthetic implants typically use stimulus levels that excite volumes of neural tissue ranging from cubic millimeters to cubic centimeters around the electrode. Because of the large stimulus intensities required, precise control of the response of neurons within the first few cell layers of an implanted electrode has not been necessary. Recent developments in the areas of micromachining and fabrication of silicon integrated circuit microelectrodes have introduced the possibility of controlled stimulation of smaller volumes of neural tissue--on the order of one thousand to one hundred thousand times smaller than those used today.

The efficiency of these microelectrodes depends on the micro-environment around stimulating sites. The surface of the microelectrodes and the proteins that adsorb to this surface have a major impact on the way in which different cell populations react to the implant. Neural growth cones are sent out from many neurons around a microelectrode following implantation. With appropriate surfaces it may be possible to get selected neurons to send processes directly to the microelectrodes. Glia and other cells also respond to an implanted electrode. With appropriate surfaces it may be possible to promote cell adhesion and anchoring of some areas of the implant structure while leaving other areas with minimal response from glial cells. This study will investigate cellular and molecular responses to specific surface modifications of silicon microelectrodes.

RESULTS

Surface Analysis and Stability Measurements

X-ray photoelectron spectroscopy (XPS) is necessary for this program in the same way that an NMR spectrometer is necessary for conducting an organic synthesis program. Since we are synthesizing surfaces and modifying their properties, we will need to assay the result of the surface before (starting material) and after (reaction product) modification. This is analogous to examining a procedure by NMR. One would not think to run an organic synthetic reaction without an examination of the product at the end of each step; in the same sense, it is crucial for us to examine the product in our surface modification experiments.

Previous work in this contract has shown that the adsorbed proteins expected in any real biological evaluation stabilize the SAM monolayer for an initial period. The reaction mechanism of the SAM with the surface is also a key element, and we have seen groups with multiple attachment points (monochloro vs. trichloro silanes) to be more stable also. Finally, the glass itself appears to be unstable in the presence of PBS; so we are now extending these experiments to other surfaces used in making microelectrodes and their passivation layers. This will be detailed in a later report.

The examination of the deposited material after culture has proved to be very informative in diagnosing the cells' response to surfaces and correlates with morphology. We have shown that in healthy cell cultures, in our model system, that the deposited material increases over a one to three day period and then stabilizes. Conversely, on surfaces where cells display undesirable morphological features such as clumping, necrosis, and cell death, the layer continues to increase until the end of the culture. We are preparing a manuscript to detail these observations.

Surface modification and cell culture

We have successfully established adult cortical cell culture in our defined *in vitro* model system. Figure 1 illustrates adult cells after one and four days in culture. We will

extend this work to further the goals of the program in determining the best surfaces to derivatize microelectrodes for *in vivo* applications. We have also instituted the detailed statistical analysis of the culture results for incorporation into a multivariant analysis program. Figure 2 illustrates a statistical compilation of astrocyte culture results that we published in quarterly reports 2 and 3 after analysis with the NIH software program NIH Image. These results clearly support the pictorial evidence that the astrocyte cultures respond differently to different growth factors on different surfaces. Figure 3 illustrates our latest efforts with microglia cultures that demonstrate two different states: the ameboid state and the activated state present on this surface. Finally we have surface analytical results that prove the viability of our modification protocol to attach biomolecules to surfaces via a covalent cross linker (Figure 4).

Collaborations

We have established a collaboration with W. Agnew at the Huntington Medical Research Institute. While we have not yet specifically modified electrodes, we have successfully modified the poly-silastic sheath that holds a series of microelectrodes in place along the spinal cord during implantation (Agnew et al., 1990). One problem we are focused on solving is that the sheath has glial scar buildup and adhesions that eventually displace the electrodes laterally (with adhesion) and vertically (due to buildup of tissue). We visited Huntington Institute after the Neuroscience meeting in November, and have planned a series of experiments. We will begin a new series of experiments once the costs of the *in vivo* work is determined. We are also examining by surface analysis some postmortem samples supplied by D. Agnew's group. These results are ongoing and will be reported at a later date.

NEXT QUARTER OBJECTIVES

- Continue work to establish adult cortical cell culture conditions
- Begin screening biologically modified SAM surfaces for cortical cell response
- Continue surface analysis of surfaces both before and after culture
- Continue surface stability experiments
- Continue screening surfaces for microglia response
- Send samples to Huntington for *in vivo* experiments as well as explore other possibilities for collaboration in this area.

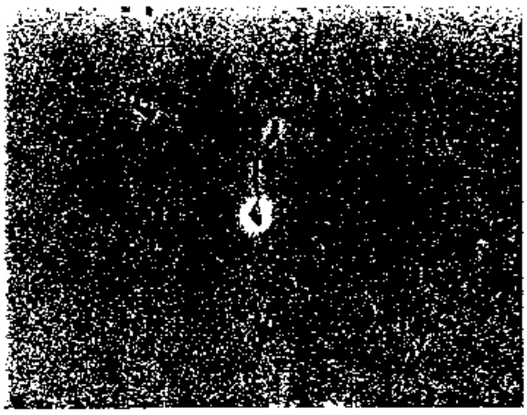
REFERENCES

Agnew, W.F., McCreery, D.B., L.A., and Yuen, T.G.H. (1990). Effects of prolonged electrical stimulation of the central nervous system. In: Agnew, WF., & McCreery, D.B. (Eds), *Neural Protheses* pp. 225-252, (Prentice Hall: Englewood Cliffs, NJ).

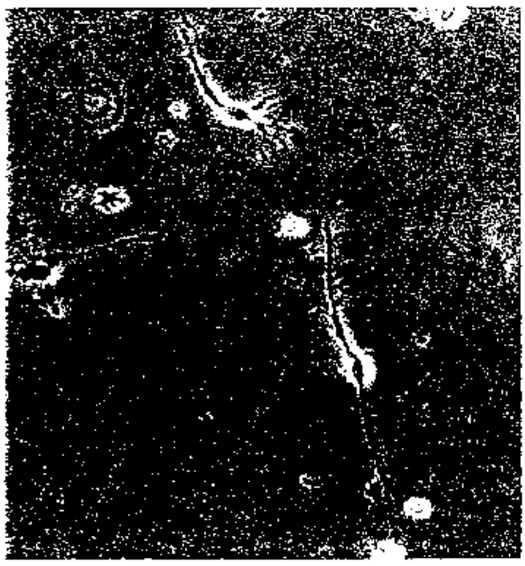
Tanghe and Wise (1992). A 16-channel CMOS neural stimulating array. *IEEE Trans. Sol State Circuits*, 27:69-75.

A.E. Schaffner, J.L. Barker, D.A. Stenger and J.J. Hickman. (1995) Investigation of the Factors Necessary for Growth of Hippocampal Neurons in a Defined System. *J.Neuroscience Methods* 62; 111-119

ADULT NEURONS OF RAT CEREBRAL CORTEX CULTURED ON DETA IN SERUM-FREE MEDIUM

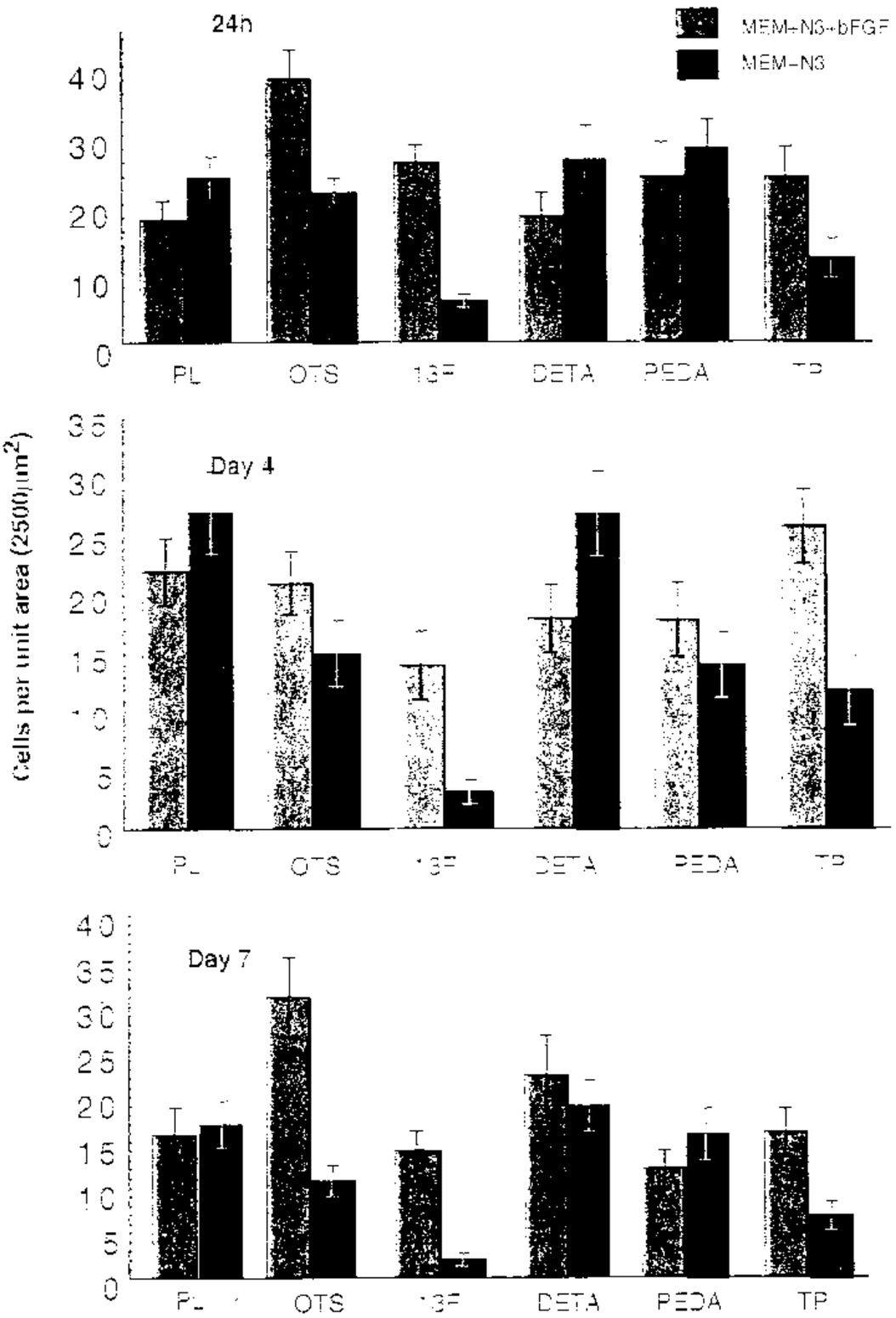


1-day-old culture



4-day-old cultures

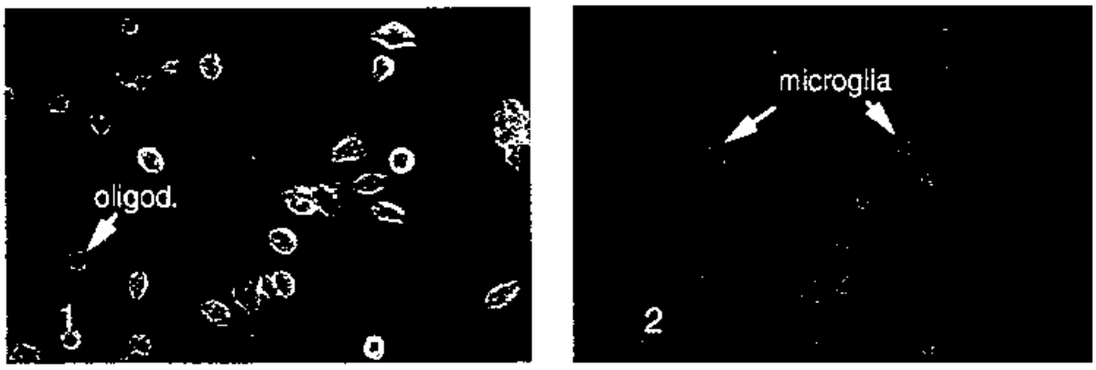
astrocytes cultured on different surfaces



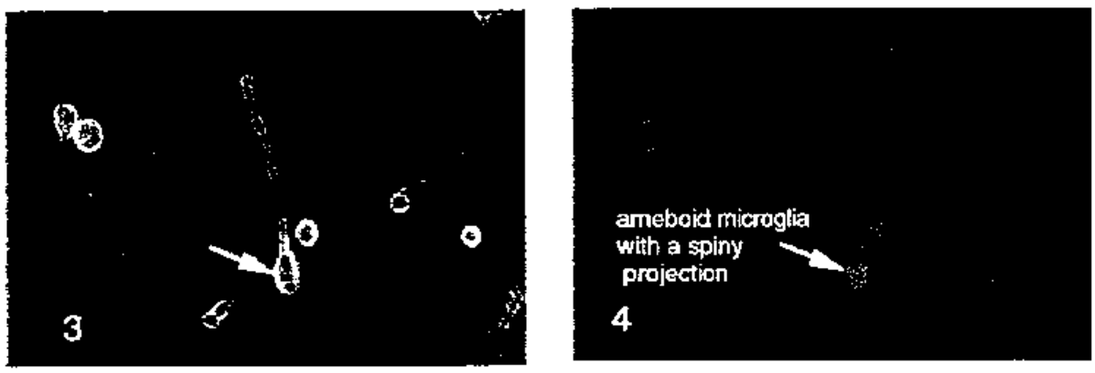
Enriched cultures of rat microglia derived from cerebral cortex

Phase-contrast

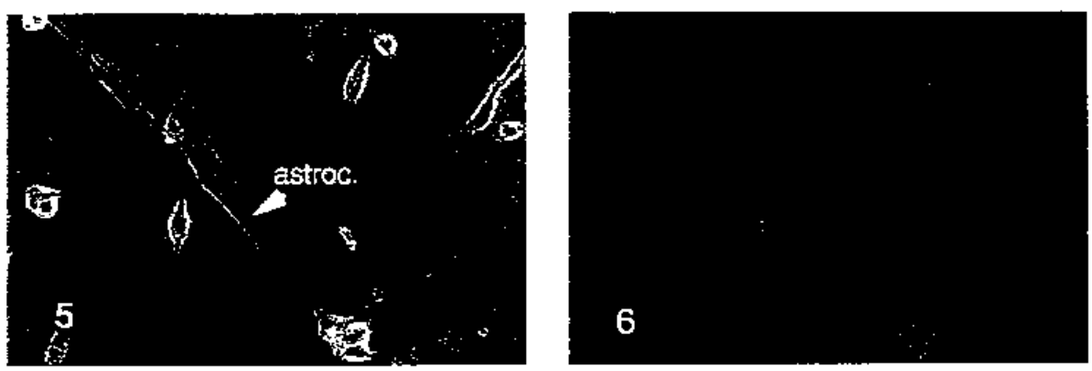
Immunostaining with Anti-OX42



Poly-D-Lysine

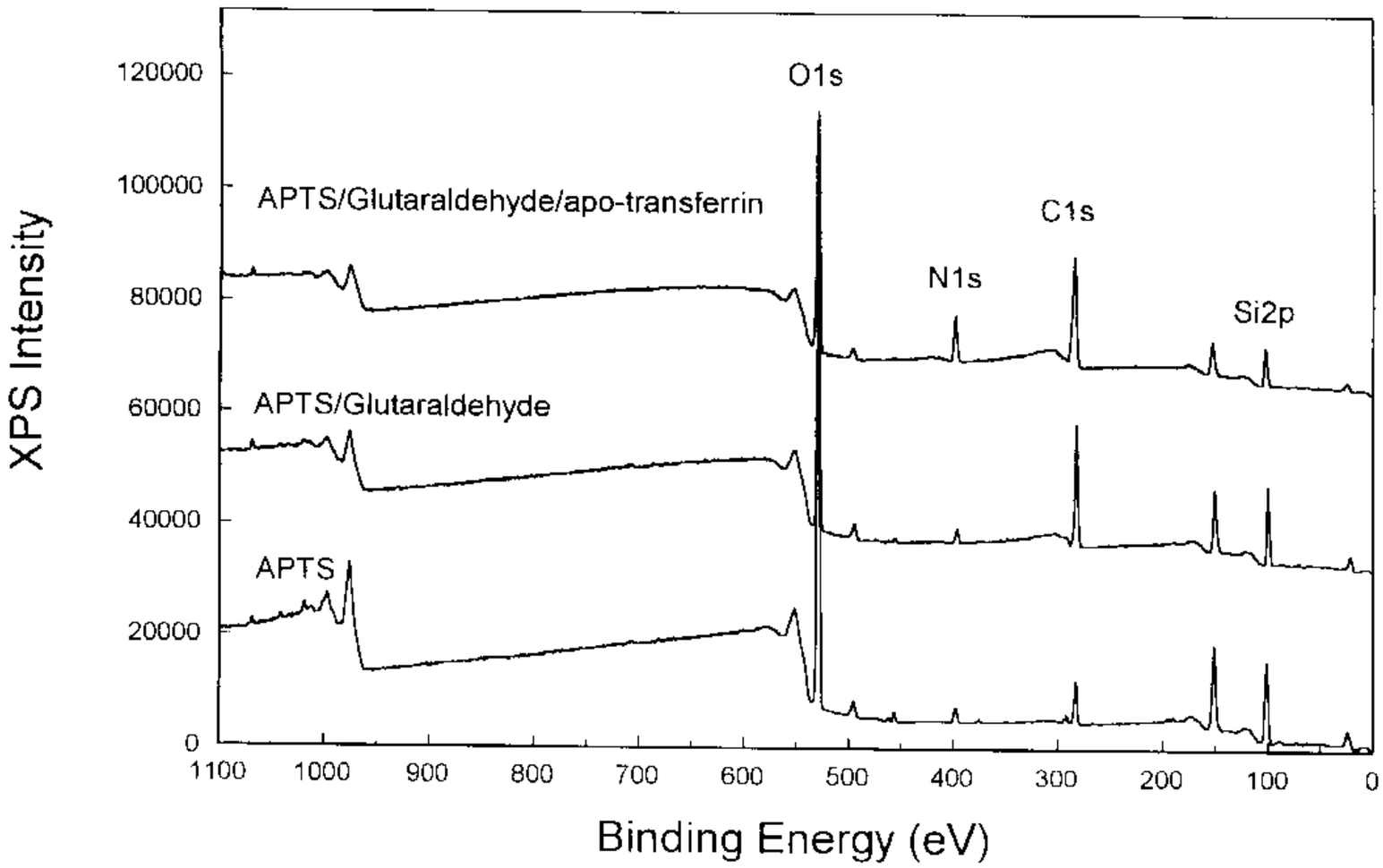


Poly-D-Lysine



DETA

Attachment of Biomacromolecules to SAMs



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